

Efficacy of nine isolates of *Beauveria bassiana* against adults of *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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Abstract: *Beauveria bassiana* (Balsamo) Vuillemin is one of the most important entomopathogenic fungi and has been widely used for many insect pests all over the world. In this study, the pathogenicity of nine isolates of *B. bassiana* was evaluated against adults of *Tribolium castaneum* (Herbst). Fifteen adults of the pest were submerged into the four suspensions (1×10^6 , 1×10^7 , 1×10^8 and 1×10^9 conidia/mL) of different isolates of *B. bassiana* for 20 s, and the mortality was recorded daily for 14 d. Results showed that IRAN 440C and IRAN 187C isolates had the lowest (5.04×10^7 conidia/mL) and highest (5.05×10^8 conidia/mL) LC_{50} , and DEBI 005 and DEBI 014 isolates had the shortest (2.88 d) and longest (4.96 d) LT_{50} , respectively. According to the LC_{50} and LT_{50} values and the mortality, IRAN 440C is a perfect isolate for using in controlling the pest.

Key words: *Beauveria bassiana*; *Tribolium castaneum*; pathogenicity; median lethal concentration (LC_{50}); median lethal time (LT_{50})

1 INTRODUCTION

The stored-product pests cause 10% to 25% annual damages to stored-products in Iran (Bagheri Zenouz, 1986). *Tribolium castaneum* (Herbst) is one of the most important stored pests that attack stored grain products such as flour, cereals, meal, crackers, beans, cake mix, dried pet food, dried flowers, chocolate, nuts, seeds, and even dried museum specimens (Via, 1999; Weston and Rattlingourd, 2000).

Phosphine has been a widely used fumigant for the control of stored-product insects for almost half century (Price and Mills, 1988; Chaudhry, 2000). It is by far the most widely used fumigant, because of its low cost, fast diffusion in air and absence of residues. These advantages contributed to an increased dependence on phosphine as fumigant (Chaudhry, 2000). However, the long-term use of a single fumigant increases the risk of resistance development in pest populations (Benhalima *et al.*, 2004). Stored product insect pests such as *Rhyzopertha dominica* (F.), *T. castaneum* (Herbst) and *Sitophilus oryzae* (L.) have been reported to develop resistance against phosphine (Sartori *et al.*, 1990). Insecticide resistance and the demand for reduced chemical inputs in

agriculture have provided an impetus to develop alternative approaches for pest control.

Biological control offers an attractive alternative or supplement to the use of chemical pesticides. Microbial biological control agents are naturally occurring organisms and perceived as being less damaging to the environment. Entomopathogenic fungi were among the first organisms to be used for the biological control of pests. More than 700 species of fungi from around 90 genera are pathogenic to insects (Hong, 2003). Most of them are found within the deuteromycetes and entomophthorales. The filamentous fungus *Beauveria bassiana* (Balsamo) Vuillemin belongs to a class of insect pathogenic deuteromycete (imperfect fungus). Fungus species of *B. bassiana* are well characterized in respect to pathogenicity to several insects and they have been used as agents for the biological control of agriculture pests worldwide (Hong, 2003). This fungus is known as the white muscardine fungus because infected insect larvae eventually turn white or grey (Duperchy, 2003).

Several studies have shown that entomopathogenic fungi *B. bassiana* and *Metarhizium anisopliae* are effective on stored products pests such as *T. castaneum*, *Sitophilus oryzae* (L.), *R. dominica* (F.) and *Acanthoscelides obtectus* Say (Padin *et al.*, 1997; Dal-Bello *et al.*, 2001; Akbar

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et al., 2004; Batta, 2004; Batta and Abu, 2005; Kavallieratos *et al.*, 2006; Michalaki *et al.*, 2006; Lord, 2010). So, nine native isolates of *B. bassiana* were used for testing their pathogenicity against adults of *T. castaneum*. Our goal was to determine the pathogenicity of different isolates of mentioned fungus against *T. castaneum*. Also, we used novel isolates that were not perused on this stored pest.

2 MATERIALS AND METHODS

2.1 Insect rearing

The red flour beetle adults were obtained from Tarbiat Modares University in Tehran/Iran and all

experiments were performed at Entomology Laboratory of Plant Protection Department of University of Maragheh in East Azarbaijan/Iran since 2011. *T. castaneum* adults were reared in plastic containers (32 cm × 22 cm × 10 cm) and their doors were covered with mesh fabric. One third of height of container was filled by wheat flour for adult feeding. These containers were maintained at $26 \pm 1^\circ\text{C}$, RH $70\% \pm 5\%$ and a photoperiod of 16L:8D for 21 days. Adults at 1 to 3 week-old were used for all bioassays.

2.2 Fungal isolates

Nine isolates of *B. bassiana* were used for this research. Characteristics of the fungi isolates are presented in Table 1.

Table 1 Isolates of *Beauveria bassiana* used in this study

Isolate	Location area	Host	Initial isolation time
EUT 116	Tehran	Larvae of wax moths	2007
DEBI 004	Ghazvin (alfalfa fields)	<i>Hyper postica</i>	2007
DEBI 005	Ghazvin (Alfalfa fields)	Tenebrionidae	2007
DEBI 014	Ghazvin (Keshtosanate hezar jolfa)	<i>Lixus incanescens</i>	2007
IRAN 187C	Ardebil	<i>Leptinotarsa decemlineata</i>	2007
IRAN 440C	Atashgah (Karaj)	Soil	2002
IRAN 441C	Saravan	<i>Rynchophorus feruginens</i>	2002
IRAN 428C	Rasht	<i>Chilo suppressalis</i>	2001
IRAN 429C	Hassan rood (Anzali)	<i>Chilo suppressalis</i>	2001

2.3 Fungal culture

Isolates of the fungus were cultured on SDAY (Sabouraud's dextrose agar yeast with 0.2% yeast extract, 1% pepton, 2% dextrose and 1.5% agar in 1 liter distilled water). The cultures were maintained at $25 \pm 2^\circ\text{C}$, RH $85\% \pm 5\%$ and a photoperiod of 16L:8D. Conidia from 21-day-old fungal cultures were used in the laboratory bioassays.

2.4 Preparation of conidial suspension

After incubation for 21 days at 25°C , mycelium and conidia were removed to a tube with a scalpel, and 20 mL sterile water was added. Suspension was vortexed to ensure an even distribution of the conidia prior to use, then was filtered through mesh fabric in order to separate mycelium and transferred to a sterile tube. The concentration of conidia was determined by counting with a haemocytometer (Paul Marienfeld GmbH and Co. KG, Germany). After the conidia were counted using haemocytometer, the main concentration was determined using the formula $Y = 5X \times 10^4$ (X = number of conidia in five squares) (Erwin, 2002). Subsequent concentrations were

determined using the logarithmic scale.

2.5 Conidial germination

For evaluation of conidial germination, 1 mL of suspension (1×10^7 conidia/mL) was dropped on SDAY in each Petri dish. The Petri dishes were then incubated at $25 \pm 2^\circ\text{C}$, RH $85\% \pm 5\%$ and a photoperiod of 16L:8D for 18 h. After this period, 1 mL of formaldehyde 0.5% was poured into Petri dishes to stop spore germination, and then germination was checked by using a microscope and results were recorded. Each experiment was replicated three times. All suspensions displayed > 85% germination of conidia and were stored at 4°C until used.

2.6 Bioassays

Fifteen adults of the pest were treated in the fungal suspension for 20 s with submerging method for each treatment and then transferred to a plastic Petri dish. The used concentrations were 1×10^6 , 1×10^7 , 1×10^8 and 1×10^9 conidia/mL. Control insects were treated with sterile distilled water. 0.01% Tween 80 (Sigma, St. Louis, MO) was added to all treatments (control and four conidial

suspensions). Treated insects were kept in incubator ($25 \pm 2^\circ\text{C}$ and RH $85\% \pm 5\%$ and a photoperiod of 16L:8D. Wheat flour was added at the 2nd day of incubation for each Petri dish. The dead adults before 24 h were removed from experiment and were kept for resolution of the fungus in Petri dish lined with moistened filter paper in the desiccators. Mortality was recorded for 14 days. The bioassay was repeated three times.

2.7 Statistical analysis

For dose-mortality bioassay, cumulative percentage mortality was normalized using arcsin transformation (SPSS Inc., 2004). The means were separated by LSD multiple range test ($P \leq 0.05$) using the ANOVA procedure of SAS (2002). Probit analysis was used to calculate the values of the lethal concentration (LC_{50}) and the lethal time (LT_{50}) for each isolate (SAS institute, 2002).

3 RESULTS

The pathogenicity of nine isolates of *B. bassiana* against adults of *T. castaneum* in the laboratory differed among treatments. Germination of

these isolates was over 85%. No mortality was detected in the control. Among these isolates, IRAN 440C and IRAN 187C exhibited the highest and lowest toxicity with the LC_{50} values of 5.04×10^7 and 5.05×10^8 conidia/mL, respectively (Table 2). The LT_{50} values for these isolates varied from 2.9 to 5 days and the shortest LT_{50} was related to DEBI 014 (Table 3).

At the concentration of 1×10^8 conidia/mL, the isolate IRAN 440C caused the highest mortality (60%) to *T. castaneum* adults ($F = 13.47$; $df = 9, 29$; $P < 0.0001$). At this concentration, three isolates, *i. e.*, DEBI 014, IRAN 428C and IRAN 429C, were at the same statistical groups. IRAN 440C isolate of *B. bassiana* caused higher mortality at most tested concentrations (Table 4).

4 DISCUSSION

Entomopathogenic fungi are being developed worldwide for the control of insect pests and some products are already available commercially (Ekesi *et al.*, 2001). Most investigators have reported that

Table 2 Bioassay results of nine isolates of *Beauveria bassiana* on adults of *Tribolium castaneum*

Isolate	Lethal concentration (conidia/mL) (95% FL)			χ^2	Slope $\pm$ SE	n
	LC_{90}	LC_{50}	LC_{10}			
116 EUT	1.57×10^9 ($7.27 \times 10^8 - 5.68 \times 10^9$)	1.13×10^8 ($6.42 \times 10^7 - 1.98 \times 10^8$)	8.17×10^6 ($2.20 \times 10^6 - 1.78 \times 10^7$)	42.4	1.12 ± 0.17 (0.78 – 1.46)	225
DEBI 004	2.89×10^9 ($1.48 \times 10^9 - 1.43 \times 10^{10}$)	5.03×10^8 ($3.04 \times 10^8 - 8.35 \times 10^8$)	8.76×10^7 ($1.79 \times 10^7 - 1.70 \times 10^8$)	18.3	1.69 ± 0.39 (0.91 – 2.46)	225
DEBI 005	1.59×10^{10} ($3.10 \times 10^9 - 4.32 \times 10^{11}$)	1.24×10^8 ($5.36 \times 10^7 - 3.49 \times 10^8$)	9.61×10^5 ($7.23 \times 10^4 - 3.63 \times 10^6$)	10.5	1.36 ± 0.42 (0.54 – 2.19)	225
DEBI 014	2.16×10^{10} ($4.13 \times 10^9 - 6.48 \times 10^{11}$)	2.21×10^8 ($9.76 \times 10^7 - 6.82 \times 10^8$)	2.27×10^6 ($2.18 \times 10^5 - 7.60 \times 10^6$)	28.2	0.64 ± 0.12 (0.41 – 0.88)	225
IRAN 187C	4.40×10^9 ($3.10 \times 10^9 - 4.32 \times 10^{11}$)	5.05×10^8 ($2.70 \times 10^8 - 1.03 \times 10^9$)	5.81×10^7 ($2.11 \times 10^6 - 1.42 \times 10^8$)	29.4	0.61 ± 0.11 (0.39 – 0.83)	225
IRAN 440C	2.77×10^9 ($8.95 \times 10^8 - 1.97 \times 10^{10}$)	5.04×10^7 ($2.43 \times 10^7 - 1.05 \times 10^8$)	9.17×10^5 ($1.31 \times 10^5 - 2.81 \times 10^6$)	40.9	0.74 ± 0.12 (0.51 – 0.96)	225
IRAN 441C	7.20×10^9 ($2.20 \times 10^9 - 1.75 \times 10^{11}$)	3.90×10^8 ($2.03 \times 10^8 - 9.22 \times 10^8$)	2.11×10^7 ($1.58 \times 10^6 - 5.73 \times 10^7$)	16.3	1.01 ± 0.25 (0.52 – 1.50)	225
IRAN 428C	7.80×10^9 ($2.34 \times 10^9 - 7.41 \times 10^{10}$)	2.22×10^8 ($1.13 \times 10^8 - 5.12 \times 10^8$)	6.31×10^6 ($1.18 \times 10^6 - 1.62 \times 10^7$)	34.6	0.83 ± 0.14 (0.55 – 1.11)	225
IRAN 429C	1.42×10^{10} ($3.36 \times 10^9 - 2.85 \times 10^{11}$)	2.87×10^8 ($1.37 \times 10^8 - 7.88 \times 10^8$)	5.82×10^6 ($7.17 \times 10^5 - 1.69 \times 10^7$)	26.9	0.76 ± 0.15 (0.47 – 1.04)	225

Table 3 Lethal time (d) of nine isolates of *Beauveria bassiana* on adults of *Tribolium castaneum*

Isolate	Lethal time (d) (95% FL)			χ^2	Slope $\pm$ SE	n
	LC ₉₀	LC ₅₀	LC ₁₀			
116 EUT	56 (5.11 – 6.42)	3.90 (3.58 – 4.16)	2.74 (2.20 – 3.09)	39.09	8.31 $\pm$ 1.33 (5.71 – 10.92)	225
DEBI 004	6.68 (5.72 – 8.85)	3.52 (3.12 – 3.89)	1.86 (1.28 – 2.27)	36.80	4.61 $\pm$ 0.76 (3.12 – 6.10)	225
DEBI 005	8.37 (6.84 – 14.22)	4.96 (4.02 – 5.82)	2.94 (1.51 – 3.73)	22.52	5.64 $\pm$ 1.19 (3.31 – 7.98)	225
DEBI 014	8.10 (6.38 – 12.13)	2.88 (2.43 – 3.32)	1.02 (0.61 – 1.38)	44.87	2.85 $\pm$ 0.43 (2.02 – 3.69)	225
IRAN 187C	7.81 (6.52 – 11.05)	4.12 (3.71 – 4.57)	2.18 (1.51 – 2.63)	32.51	4.62 $\pm$ 0.81 (3.03 – 6.21)	225
IRAN 440C	4.88 (4.41 – 5.73)	3.18 (2.90 – 3.44)	2.07 (1.67 – 2.36)	50.67	6.88 $\pm$ 0.97 (4.99 – 8.77)	225
IRAN 441C	8.08 (6.66 – 11.12)	3.22 (2.74 – 3.65)	1.29 (0.80 – 1.69)	46.74	3.21 $\pm$ 0.47 (2.29 – 4.13)	225
IRAN 428C	7.22 (6.31 – 9.13)	4.42 (4.07 – 4.81)	2.71 (2.15 – 3.09)	42.14	6.01 $\pm$ 0.93 (4.20 – 7.83)	225
IRAN 429C	8.56 (5.43 – 109.68)	3.16 (1.68 – 4.64)	1.17 (0.05 – 1.98)	14.27	2.96 $\pm$ 0.78 (1.43 – 4.50)	225

Table 4 Mortality of *Tribolium castaneum* adults caused by nine isolates of *Beauveria bassiana* at the concentration of 1×10^8 conidia/mL

Isolate	Mortality (%)
IRAN 440C	60.00 $\pm$ 7.70 a
DEBI 005	51.11 $\pm$ 4.44 ab
EUT 116	48.89 $\pm$ 4.44 ab
DEBI 014	42.22 $\pm$ 5.88 b
IRAN 429C	42.22 $\pm$ 4.45 b
IRAN 428C	42.22 $\pm$ 2.22 b
IRAN 441C	24.45 $\pm$ 2.22 c
IRAN 187C	17.78 $\pm$ 5.88 cd
DEBI 004	15.55 $\pm$ 2.22 cd

treatment of stored grain pests with entomopathogenic fungi, especially *M. anisopliae* and *B. bassiana* can be effective (Hidalgo *et al.*, 1998; Kassa *et al.*, 2002; Batta *et al.*, 2005). The results of the present study indicated that although all the tested isolates were pathogenic and caused mortality in *T. castaneum*, they had different virulence.

T. castaneum larvae that were fed for 24 h on flour contaminated with *B. bassiana* conidia showed significantly less consumption and weight compared to those fed on fungus-free flour. Thus, the presence of

B. bassiana conidia on flour may cause a dietary stress (Lord, 2010). Akbar *et al.* (2004) tested the ability of *B. bassiana* alone and in combination with diatomaceous earth against *T. castaneum*. Their results showed that diatomaceous earth had high efficacy for controlling the pest. Wakefield *et al.* (2005) demonstrated that some *B. bassiana* isolates can caused 100% mortality in *Oryzaephilus surinamensis*, *Ephestia kuehniella*, *Lepinotus patruelis* and *Acarus siro* 10 days after treatment at 1×10^8 conidia/mL. Adane *et al.* (1996) indicated that several isolates of *B. bassiana* tested against *Sitophilus zeamais* (Motsch.) adults showed pathogenicity to insects, but there were highly significant differences among the isolates with respect to virulence. These isolates caused 37% to 100% mortality in *S. zeamais*. They also had recorded the medium lethal time for different isolates. The lowest time (2.74 days) was recorded for isolate 189-481.

Cherry *et al.* (2005) also have demonstrated that different isolates from *M. anisopliae* and *B. bassiana* could provide good control of *Callosobruchus maculatus* by immersion bioassay. They represented that the LT₅₀ value for *B. bassiana* and *M. anisopliae* isolates varied from 3.11 to 6.13 days (with an average of 4.61 days) and 3.27 to 5.62 days (with an average of 4.60 days). On the contrary, other investigators have

reported that treatment of *S. oryzae* on wheat grains with *M. anisopliae* alone was not effective, but that 50% adult mortality of the pest was achieved at 30 d after treatment with a conidial suspension mixture of *M. anisopliae* and *B. bassiana* (Dal-Bello *et al.*, 2001).

According to our results, the isolate IRAN 440C is the most pathogenic to adults of *T. castaneum*, so this isolate is appropriate for controlling this pest. Our research suggested that the entomopathogenic fungi such as *B. bassiana* are perfect microbial agents for control of stored products pest. However, further studies need to be done on formulating and improving application methods.

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球孢白僵菌九个菌株对赤拟谷盗成虫的毒效

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摘要: 球孢白僵菌 *Beauveria bassiana* (Balsamo) Vuillemin 是最重要的昆虫病原真菌, 广泛用于防治世界各地的多种害虫。本研究评价了球孢白僵菌 9 个菌株对赤拟谷盗 *Tribolium castaneum* (Herbst) 成虫的致病性。将 15 头赤拟谷盗成虫浸入到 4 个浓度 (1×10^6 , 1×10^7 , 1×10^8 和 1×10^9 个分生孢子/mL) 的白僵菌菌株中 20 s, 14 d 内每日记录成虫的死亡率。结果表明: IRAN 440C 菌株对赤拟谷盗成虫的 LC_{50} 最低 (5.04×10^7 个分生孢子/mL), IRAN 187C 菌株的最高 (5.05×10^8 个分生孢子/mL); DEBI 005 菌株对赤拟谷盗成虫的 LT_{50} 最短 (2.88 d), DEBI 014 菌株的最长 (4.96 d)。根据 LC_{50} , LT_{50} 和死亡率结果得出 IRAN 440C 是防治这一害虫的理想菌株。

关键词: 球孢白僵菌; 赤拟谷盗; 致病性; 致死中浓度 (LC_{50}); 致死中时 (LT_{50})

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